

EcoRI **ClaI** **PvuII** **BamHI**

1.5 kb

5 3 4

P RBS PRE PRD MAT TERM

1 GGCTACTAAATATTATCCATACATACTAATTAATACACAGAAATCTGCTCTATTGGTTATTCGCAATGAAAAAGGAGGATAAAGA GTG

Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe Gly Ser Thr Ser
99 AGA GGC AAA AAA GTA TGG ATC AGT TIG CTG TTT GCT TTA GCG TTA ATC TTT ACG ATG GCG TTC GGC AGC ACA TCC

-100 PRE -90 -60

174 TCT GCC CAG GCG GCA GGG AAA TCA AAC GGG GAA AAG AAA TAT ATT GTC GGG TTT AAA CAG ACA ATG AGC ACG ATG

Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met
174 TCT GCC CAG GCG GCA GGG AAA TCA AAC GGG GAA AAG AAA TAT ATT GTC GGG TTT AAA CAG ACA ATG AGC ACG ATG

-50 -40 -30

249 AGC GCC GCT AAG AAG AAA GAT GTC ATT TCT GAA AAA GGC GGG AAA GTG CAA AAG CAA TTC AAA TAT GTA GAC GCA

Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala
249 AGC GCC GCT AAG AAG AAA GAT GTC ATT TCT GAA AAA GGC GGG AAA GTG CAA AAG CAA TTC AAA TAT GTA GAC GCA

Ala Ser Ala Thr Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp
324 GCT TCA GTC ACA TTA AAC GAA AAA GCT GTA AAA GAA TTG AAA AAA GAC CCG AGC GTC GCT TAC GTT GAA GAA GAT

-107 Met

FIG.-1A

2/6

-1 | 1
MAT

His Bal Ala His Ala Tyr Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln
399 CAC GTA GCA CAT GCG TAC GCG CAG TCC GTG CCT TAC GGC GTA TCA CAA ATT AAA GCC CCT GCT CTG CAC TCT CAA

20 30 40
Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asn Leu Lys Val
474 GGC TAC ACT GGA TCA AAT GTT AAT GGT AAA GTA GCG GTT ATC GAC AGC GGT ATC GAT TCT TCT CAT CCT GAT TTA AAG GTA

50 60
Ala Gly Gly Ala Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala
549 GCA AGC GGA GCC AGC ATG GTT CCT TCT TCT GAA ACA AAT CCT TTC CAA GAC AAC AAC TCT CAC GGA ACT CAC GTT GCC

70 80 90
Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys
624 GGC ACA GTT GCG GCT CTT AAT AAC TCA ATC GGT GTA TTA GGC GTT GCG CCA AGC GCA TCA CTT TAC GCT GTA AAA

100 110 120 130 140
Val Leu Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn Asn Met
699 GTT CTC GGT GCT GAC GGT TCC GGC CAA TAC AGC TGG ATC ATT AAC GGA ATC GAG TGG GCG ATC GCA AAC AAT ATG

150
Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
774 GAC GTT ATT AAC ATG AGC CTC GGC GGA CCT TCT GGT TCT GCT GCT TTA AAA GCG GCA GTT GAT AAA GCC GTT GCA

160
Ser Gly Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Thr Val Gly Tyr Pro Gly
849 TCC GGC GTC GTA GTC GTT GCG GCA GCC GGT AAC GAA GGC ACT TCC GGC AGC TCA AGC ACA GTG GGC TAC CCT GGT

FIG-1B

FIG.-1C

**CONSERVED RESIDUES IN SUBTILISINS FROM
*BACILLUS AMYLOLIQUEFACTENS***

1	A	Q	S	V	P	.	G	A	P	A	.	H	.	.	G	20
21	.	T	G	S	.	V	K	V	A	V	.	D	.	G	H	P	40
41	D	L	.	.	.	G	G	A	S	.	V	P	Q	D	60
61	.	N	.	H	G	T	H	V	A	G	T	.	A	A	L	N	N	S	I	G	80	
81	V	L	G	V	A	P	S	A	.	L	Y	A	V	K	V	L	G	A	.	G	100	
101	S	G	.	.	S	.	L	.	.	G	.	E	W	A	.	N	120	
121	V	.	N	.	S	L	G	.	P	S	.	S	A	.	.	140	
141	G	V	.	V	V	A	A	.	G	N	.	G	.	.	.	160	
161	Y	P	.	.	Y	A	V	G	A	.	180	
181	D	.	.	N	.	.	A	S	F	S	.	.	G	.	.	L	D	.	.	A	200	
201	P	G	V	.	.	Q	S	T	.	P	G	.	.	Y	.	.	.	N	G	T	220	
221	S	M	A	.	P	H	V	A	G	A	A	A	L	.	.	.	K	.	.	.	240	
241	W	.	.	.	Q	.	R	.	.	L	.	N	T	.	.	.	L	G	.	.	260	
261	.	.	Y	G	.	G	L	.	N	.	.	A	A		

FIG. 2

Comparison of subtilisin sequences from:

B. amyloliquefaciens
B. subtilis
B. licheniformis
B. lentus

01	10	20	30	
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P				
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P				
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P				
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T A H P				
41	50	60	70	
D L K V A G G A S H V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G				
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G				
D L N V V G G A S F V A G E A Y N A T D G N G H G T H V A G T V A A L D N T T G				
D L N I R G G A S F V P G E A P S T Q D G N G H G T H V A G T I A A L N N S I G				
81	90	100	110	
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D				
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D				
V L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T N G M D				
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N G M H				
121	130	140	150	
V I N M S L G G P S S G S A A L K A A V D K A V A S G V V V A A A A G N E G T S G				
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V A A A A G N E G S S G				
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V A A A A G N S G S G				
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V A A A S G N S G A G S				

FIG 3/A

161 S S S T V G Y P G K Y P S S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
S S T V G Y P A K Y P S S T I A V G A V D S S N Q R A S F S S A G S E L D V M A
S T N T I G Y P A K Y P S S V I A V G A V D S S N Q R A S F S S V G A E L E V M A
* * * I S Y P A R Y A N A H A V G A T D Q N N R A S F S Q Y G A G L D I V A
170
180
190
201 P G V B I Q S T L P G C N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N
P G V S I Q S T L P G C T Y G A Y N G T S M A T P H V A G A A A L I L S K H P T
P G A G V Y S T Y P C S T Y A S L N G T S M A T P H V A G A A A L I L S K H P N
P G V N V Q S T Y P C S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S
210
220
230
241 W T N T Q V R S S L E N T T A T Y L G S S F Y Y G K G L I N V Q A A A Q
W T N A Q V R R L E S S T A T Y L G S S F Y Y G K G L I N V Q A A A Q
L S A S Q V R R L E S S T A T Y L G S S F Y Y G K G L I N V Q A A A Q
W S N V Q I R N H L K N T A T S L G S S T N L Y C S S G L V N A E A A T R
250
260
270

FIG 3/B

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